PROJECT NUMBER: 1620
PROJECT TITLE: Electrophysiological Studies
PROJECT LEADER: F. P. Gullotta
WRITTEN BY: C. S. Hayes
PERIOD COVERED: May, 1989

## I. NASAL EVENT-RELATED POTENTIALS (NERPs)

A. **Objective**: To develop methods to objectively and reliably evaluate human responses to smoke constituents and tobacco flavorants.

B. **Results**:

Cognitive NERP Study

At the request of Flavor Development, subjective testing was conducted on four menthol samples for Project LEVO. No statistically significant differences between each of the four submitted samples and a standard were observed.

In collaboration with Dr. Ken Mullen (Visiting Scientist - PED), target and standard data from the natural vs synthetic menthol discrimination were subjected to principal component and step-wise discriminant analyses. Preliminary findings suggest that discriminant analysis may be a very powerful statistical tool for differentiating between target and standard NERPs.

C. **Plans**: Resume NERP testing of various synthetic and natural menthol substitutes for Flavor Development as requested. Complete testing of ten subjects in the 75:25 synthetic/natural menthol mixture vs synthetic menthol comparison. Utilize the step-wise discriminant analysis technique to analyze the data from the natural/synthetic menthol mixture vs synthetic menthol comparison.

PROJECT NUMBER: 1702
PROJECT TITLE: Optical Processing and Aerosol Research
PROJECT LEADER: K. A. Cox
PERIOD COVERED: May, 1989

## I. CUT CLOSURE STAMP INSPECTION SYSTEM

A. **Objective**: Develop and implement a system for the off-line QA inspection of cut closure stamps.

B. **Results**: A device designed to detect the occurrence of double stamp feeds was assembled and tested for reliability. The device worked well for all stamp types currently under consideration. Efforts to enhance the inspection algorithm to permit the reliable inspection of white stamps in the presence of lighting fluctuations have been initiated.

C. **Plans**: Complete enhancement and testing of the inspection system. Transfer to QA for evaluation.

## II. HIGH SPEED PRINT INSPECTION

A. **Objective**: Develop methods for the inspection of print on a printing press.

B. **Results**: For very high speed (200-400 images/sec) implementation of the total image inspection algorithm, it is useful to simplify the filter (discriminant image). This reduces the computational effort needed for the execution of the algorithm as well as that needed for the generation of the filter. One way to achieve this simplification is to restrict the filter to have a binary structure (rather than floating point). This not only reduces computational requirements, but also simplifies image acquisition and interfacing. An algorithm has been developed for generating such a binary filter. The algorithm is adaptive and superior to the common procedure of simply truncating a floating point filter. A binary filter was generated using a training sample size of 300 good images and tested on a separate test set. The algorithm worked well. Further improvements in inspection capability were obtained when the image was segmented and an inspection carried out on each segment.

C. **Plans**: Carry out further testing of the adaptive inspection algorithm. Identify hardware architectures suitable for very high speed implementation

## III. INDIVIDUAL CIGARETTE INSPECTION

A. **Objective**: Develop methods for the on-line inspection of individual cigarettes.

B. **Results**: Estimates were obtained for the light requirements of a linear photocell array, and on the availability of suitably fast photocell arrays. In principle, on-line viewing of cigarettes with a linear photocell array can provide data with more linearity and freedom from distortion than a scanning device. A Reticon photocell capable of operating with an exposure time as small as 4 µsec has been located. This is more than adequate for our 10-µsec requirement. Although the available driving circuit limits the array to operation at a 28-µsec exposure time, Reticon claims a faster driving circuit can be developed and may already exist in a customer facility.

The light requirement for the array is not expected to be a problem. Fast inspection systems using this array are currently employing halogen lighting systems.

C. **Plans**: Evaluate the Reticon system in parallel with the AO scanner due to be delivered in early June.

## IV. AEROSOL GENERATION

A. **Objective**: Develop and characterize a laboratory aerosol generator capable of producing highly concentrated aerosols.

B. **Results**: The aerosol generation system is now operational. Glycerine aerosols have been generated with mass concentrations and particle sizes similar to that of the tobacco smoke aerosol. Attempts to generate a water aerosol, however, have been unsuccessful. The high diffusivity of water vapor may account for this result. The water appears to condense on the walls of the cooling section rather than form an aerosol. A new cooling section incorporating a porous, metal tube has been fabricated. With this system, cool gas will flow through the walls of the tube and mix with the hot, vapor-laden gas from the furnace. This system is expected to be better characterized, allow greater control over the cooling rate, and have reduced condensation at the walls.

C. **Plans**: Test the aerosol generator incorporating the porous tube.

PROJECT NUMBER: 1704
PROJECT TITLE: Supercritical Fluid Processes
PROJECT LEADER: T. M. Howell
PERIOD COVERED: May, 1989

I. LOW NICOTINE

A. Objective: Develop second generation processing for ART.

B. Results: The Separations Research Program, University of Texas at Austin, was contracted to model flooding velocities and mass transfer efficiencies for a number of different contacting devices in two different modes of operation, continuous water phase and continuous $CO_2$ phase. The data they presented suggested that using Intalox 2T column internals in a continuous water phase mode would provide for the most efficient scrubbing of nicotine from the extractive carbon dioxide phase. Verification of this work is scheduled to be performed at the General Foods facility at Hoboken.

Units are being fabricated for obtaining additional $CO_2$-water-nicotine equilibrium data vital to the water absorber design and for evaluating a tray design to be used in a proposed batch liquid absorber concept.

C. Plans: Work is on going.

II. LOW NICOTINE

A. Objective: Support ART commercial plant.

B. Results: As reported last month, it was considered that the presence of tobacco solubles in any free water within the extraction system would decrease the solubility of oxygen in that water. Analysis of drain samples from the BHPP did not show any significant change in oxygen concentration from pure water when analyzed under the same oxygen-containing atmosphere.

The distribution coefficient for oxygen between vapor phase $CO_2$ and liquid $CO_2$ at 800 psig and 19°C was calculated to be approximately 4.9. This number was verified by Dr. Teja of Georgia Tech.

Analytical Research was asked to quantitate the amount of interstitial oxygen in tobacco and its rate of diffusion out of the filler with and without a vacuum applied. Procedures for this determination are in the development stages.

C. Plans: Work is ongoing.

## III. LOW NICOTINE

A. **Objective**: Evaluate alternate methods of nicotine extraction.

B. **Results**: A gas mixture of 75% nitrogen and 25% carbon dioxide wt/wt at 260 bar and 60°C was used to extract DL Blend filler (25% OV, 3% AB). Nicotine reductions of 9% and 3% were achieved using ART stems and water, respectively, as absorbers and 200 M/M of the gas mixture. (Reference German patent DE 3706595.5 by Dr. Hans Gahrs).

C. **Plans**: No further work is anticipated.

PROJECT NUMBER: 1708
PROJECT TITLE: Physical Chemistry and Process Monitoring
PROJECT LEADER: J. L. Banyasz
PERIOD COVERED: May, 1989

I. OPERATIONS SUPPORT (D. Driscoll in collaboration with the Applied Technology Group)

A. Objective: Characterization of side seam adhesive application.

B. Results: The results obtained with simulator studies have indicated that side seam adhesive flow varies as a function of adhesive level in the reservoir, reservoir size and configuration, as well as nozzle size. As a result, the group has developed and installed a flow controller on the simulator which controls the flow at a predetermined level.

C. Plans: Flow measurements will next be carried out in the factory. Optimal flow rates as a function of maker speed will ultimately be determined.

II. MENTHOL STUDIES (T. V. Van Auken)

A. Objective: Determine the diffusion rate and solubility of menthol in cellulose acetate.

B. Results: The study of menthol sorption by filter tow as a function of moisture and plasticizer content has been completed. Water, menthol, and triacetin were found to migrate in the filter plugs. The results indicate that high moisture levels facilitate the migration of both menthol and triacetin. Triacetin appears to increase the menthol sorption capacity of CA but lowers its ability to hold moisture.

C. Plans: Experiments with high moisture conditions will be continued to determine optimum conditions for loading CA with menthol.

III. OPERATIONS SUPPORT (P. Henderson in collaboration with the Applied Technology Group)

A. Objective: Characterization of inks.

B. Results: Work has been initiated to extend the data base to other brands of monogram ink.

C. Plans: This work is ongoing.

IV. LOW DENSITY RODS (S. Ganeriwala)

A. **Objective**: Compare compression properties of low density and control rods.

B. **Results**: Compression measurements on low density and control rods as a function of RH are continuing. Compression tests were also run on ART cigarettes using Merit as a control. The preliminary results indicate no differences. Further tests are under way.

PROJECT NUMBER: 1720
PROJECT TITLE: Analytical Microscopy
PROJECT LEADER: V. L. Baliga
PERIOD COVERED: May, 1989

## I. LOW SIDESTREAM CIGARETTE PAPERS (SANDERS, BALIGA)

### A. Objective:

Examine the ultrastructure of selected cigarette papers and paper additives in support of the low sidestream project.

### B. Results:

Sol Gel: Several sol gel samples were examined for size, structure, and elemental composition. Sample #8741-91-162-1 had been prepared with ethanol, dried at 200°C, and ground. The gross particle size measured 1μm to 12μm long with an aspect ratio that ranged from 1 to 8. The large particles were stacks of rectangular platelets, 0.2μm in length, which seemed to flow together. The platelets consisted of unit particles which measured less than 0.01μm. Aluminium was the only element detected[1].

The gross structure of sol gel #8741-100-171-1P consisted of angular particles which were less than or equal to 60μm in length. Two structures were noted that appeared to be unique particles. One was an elongated particle that measured 0.2μm in length and a rounded particle that measured 0.01μm to 0.1μm in diameter. The samples contained Al with a small amount of Cl[1].

Two samples of sol gel, synthesized by Dr. Schleich, were examined. The gross structure of sample #8741-117-1 measured 10μm to 150μm along the longest axis. The particles were rough textured and consisted of smaller and smaller particles with a unit particle which measured 0.03μm to 0.1μm in diameter. Elements present were Al and S. Sample # 8741-117-2 consisted of large angular particles with finely textured surfaces. The large particle size was no greater that 600μm while the tightly packed unit particles were 0.03μm to 0.1μm in diameter. Al was present in this sample[2].

Two alumina sol gel samples which had been precipitated at different pHs, contained a variety of sizes of angular structures that were less than or equal to 1.7mm long. Sample #8741-103-1A consisted of unit particles which measured 0.01μm to 0.02μm in diameter. The unit particles which formed sample # 8741-103-2A were consistently smaller and finer than those in sample #8741-103-1A. Al was the only element found in both samples[3].

A calcium aluminate sample contained aggregates of particles which measured to 180μm in diameter. Particles within the aggregate contained unit particles that were irregular in shape and measured 0.01μm to 0.27μm in diameter. Elements present were Al and Ca with a small amount of Cl[3].

Commercial samples were examined. The $Al(OH)_3$ by Reheis Chemical Co. contained large rounded aggregates which consisted of unit particles that measured 0.01μm to 0.08μm in diameter. Elements that were present included Al with lesser amounts of Na, Cl, and Ca[1]. A sol gel prepared by Union Carbide was in the form of spheres with differing degrees of hollowness. The unit structure of this sample was equant in shape and measured 0.03μm to 0.1μm in diameter[4].

Other Chemical Additives: Two samples of hydrotalcite [$Mg_6Al_2(OH)_{16}CO_3 \cdot 4H_2O$] were examined, one with a vanilla flavorant and one without. The plain hydrotalcite consisted of rounded aggregates of smaller spherical aggregates. This rounded shape was carried through to the unit particle which measured 0.03μm to 0.1μm in diameter. The hydrotalcite with vanilla flavorant consisted of two types of crystals. One was similar to that of the plain hydrotalcite while the other was very angular with multiple faces. This structure contained Al only. The spherical, rough textured material contained both Mg and Al[5].

C. **References**:


1. Sanders, K., "Analysis of Reheis Chemical Co. and Sol Gel Samples," Memo to A. Kallianos, April 21, 1989.
2. Baliga, V., "Characterization of Sol Gels (Schleich)," Memo to A. Kallianos, May 2, 1989.
3. Sanders, K., "Analysis of Alumina and Calcium Aluminate Samples," Memo to A. Kallianos, May 12, 1989.
4. Baliga, V., "Structural and Elemental Characterization of Boehmite Alumina," Memo to T. Sanders, May 3, 1989.
5. Baliga, V., Structural and Elemental Characterization of Experimental Chemical Additives," Memo to J. Paine, April 28, 1989.


II. ART (SANDERS)

A. **Objective**: Examine samples for the continuing research and development efforts of the ART Pilot Plant.

B. **Results**: Four sets of stems - control CRS stem with citrate; control CRS stem; ART stem, top of basket; and ART stem, bottom of basket were examined. These stems were water washed and dried and compared as were stems that had citrate added to them at a later time. Also examined was RL made from the above stems and a sample of monopotassium citrate. The following conclusions were drawn. Elements normally found in stem material leach from the cellular matrix and redeposit on the surface during citrate addition and stem processing. These deposits can be resolubilized and removed from the stem by water washing or by the addition of citrate solution. If the stem is dried in a manner which allows the solution to drip away from the stem, a greater proportion of the original elemental constituents of the stem are removed versus being reabsorbed into the stem. There was a difference in the amount and form of redeposited material between ART stem from the top versus the bottom of the basket. During the pressure let-down operation in the ART

process, the platelet crystals found on stem from the top of the basket were removed while the globular material found on stem from the bottom of the basket remained. The redeposited crystals were subsequently removed during processing of the stem into RL. Normal morphology of the ART processed stems and those on the RL sheet was maintained when compared against control stems. Normal morphology was also maintained after water washing and drying the stems[1].

C. References:

Sanders, K., "Analysis of Control and ART Stems and RL made from Control and ART Stems," Memo to B. Handy, May 9, 1989.

## III. RESPONSE TO ANALYTICAL REQUESTS (SANDERS, BALIGA)

A. Objective: Provide analytical support to R&D.

B. Results:

Two stainless steel blades used to cut filler to 30 cuts per inch were examined. The blade sharpened with a coarse stone contained larger grooves and ridges than the blade sharpened with a finer grinding stone[1].

A customer complaint cigarette was examined for foreign materials. None were found[2].

Tobacco dust from Cabarrus was examined for the presence of glass. No glass was found in the sample received[3].

C. References:

1. Sanders, K., "Analytical Microscopy Request Form," to S. Zimmermann, May 11, 1989.
2. Baliga, V., PM Notebook #8412, p. 178.
3. Baliga, V., PM Notebook #8412, p. 178.

## IV. SAFETY (SANDERS)

A. Objective: Serve as the R&D First Aid Team Captain and member of the Emergency Response Team.

B. Results: Emergency Medical Technician classes are being taught on a weekly basis. Training exercises at Blackstone Fire Training Center and the 1989 Cardiac - EMT Symposium were attended.

PROJECT NUMBER: 1752
PROJECT TITLE: Molecular Structure Determination
PROJECT LEADER: G. Vilcins
PERIOD COVERED: May, 1989

## I. MEASUREMENT OF OXYGEN

A. **Objective**: To determine the partition coefficients of oxygen distributed between the aqueous and gaseous phases of ART pilot plant drain water under different experimental conditions.

B. **Results**: The concentrations of dissolved oxygen in pilot plant drain water were measured by polarography using a Clark electrode. Oxygen solubility was determined for samples purged with air, 80% carbon dioxide-20% air, and pure carbon dioxide. Reference solutions consisted of 20% propylene glycol, 20% propylene glycol-0.8% glycerine, 5% glycerine, 1% sodium chloride, and 4% sodium chloride solutions in water.

C. **Conclusions**: The concentrations of dissolved oxygen in the ART pilot plant drain water and reference solutions decreased in proportion to decreased levels of oxygen in the purge gas.

D. **Plans**: The analysis of the data is in progress.

E. **References**:

1. Howell, T., "Oxygen Solubility in Pure Water Versus ART Pilot Plant Drain Water Containing Tobacco Solubles," memo to E. B. Fisher, April 4, 1989.
2. Shafer, K., PM Notebook #8789, p. 10

## II. MEASUREMENT OF ABSORBANCE AND SCATTERING OF LIGHT BY SMOKE IN THE VISIBLE REGION

A. **Objective**: To ascertain the relative contributions of light scattering and absorption of smoke in the visible region (400-700 nm).

B. **Results**: Absorbance of TPM films, fresh whole smoke, aged whole smoke, and gas phase smoke were measured over the entire visible region using a Beckman DU-6 UV-Visible spectrophotometer. The change in absorbance of whole smoke with aging also was monitored at selected wavelengths.

C. **Conclusions**: These results showed that the absorbance/scattering behavior was attributed to those particles in smoke greater than 0.3 microns in size. That scattering is the principal effect was demonstrated by the fact that changes in transmittance of smoke with time were similar whether the entire visible region or a selected region was scanned.

D. **References**:

Jensen, N.,"Measurement of Absorbance and Scattering of Light by Smoke in the Visible Region," memo to J. Whidby, May 11, 1989.

**PROJECT NUMBER:** 1757
**PROJECT TITLE:** Analytical Flavor Specifications
**PROJECT LEADER:** M. L. Zimmermann
**PERIOD COVERED:** May, 1989

## ANALYTICAL FLAVOR SPECIFICATIONS

A. **Objective**: To develop analytical and sensory specifications for incoming flavors and materials for use at the Flavor Center and other QA facilities. To provide analytical certification on export flavors manufactured at the Flavor Center for FRG compliance.

B. **Results**:

The analysis of Direct Materials for compliance to FRG continues to require a large commitment of time. We are working in conjunction with Purchasing to certify on a priority basis those materials scheduled to be ordered. In addition, revised and preshipment samples are also received and analyzed following the samples for Purchasing to determine acceptability from a certification standpoint.

The PMI sample is being formulated again and samples of specific lots of materials for each of the the pre-blends will be analyzed.

Flavors currently on hand at the Flavor Center are being examined, with the Flavor Center doing a preliminary screening process. We are receiving approximately 30 additional samples per week by this process for quantitative confirmation.

Flavor samples for the other special projects were also analyzed and over seventy-five percent were found to be contaminated at various levels.

C. **Plans**: Continue the certification of the PMI samples, begin the certification of all flavors currently on hand at the Flavor Center and continue the specification work for the second vendor.

PROJECT NUMBER: 1759
PROJECT TITLE: Materials Evaluation & Elemental Analysis
PROJECT LEADER: P. F. Grantham
PERIOD COVERED: May, 1989

## I. MATERIALS EVALUATION

A. **Objective**: To identify components of commercial products prior to their use at PM facilities.

B. **Results**:

Presentations on the Materials Evaluation program were made at Universal Leaf Tobacco (ULT) headquarters and five of their processing facilities (Thorpe Greenville, TPI, K.R. Edwards, J.P. Taylor, and Southern Processors). These presentations outlined the program and stressed the importance of eliminating the use of undesirable materials in and around the processing of our tobacco. A tour was conducted at each of these processing locations. A presentation was also made for the EWT (Employees Working Together) Group at Stockton Street.

Tours were conducted at 20th Street, BL Plant and the Leaf Processing Facility. Routine analysis of commercial products and Packaging Development samples continued.

D. **Plans**: We will assist ULT in setting up a modified Materials Evaluation program of their own. They will compile a list of their commercial products, which we will compare with our database. We will notify them of the recommendations based on PM guidelines. ULT materials which have not been evaluated at PM will be submitted through our program for analysis as time permits. We will also provide advice on conducting tours at their facilities regarding the types of materials and use/misuse of materials that they should be aware of.

## II. ELEMENTAL ANALYSIS

A. **Objective**: To provide qualitative and quantitative elemental data on tobacco, cigarette paper, development and materials evaluation samples.

B. **Results**:

X-ray fluorescence (XRF) was used to determine elemental concentrations of primarily, Mg, Ca, K, and Al in paper samples. Many of these samples were also analyzed using atomic absorption (AA). Results from these will be used in the evaluation of the new XRF paper method.

Certified, polycarbonate thin film standards were evaluated for use as standards for the paper method. The thin polycarbonate

films did not work well as standards for paper. Well characterized paper will continue to be used as standards.

Routine tobacco samples, including high K stems, sheet material, and leaf were analyzed this month using both XRF and AA.

A method was developed for determining potassium in paper using an autotitrator with a potassium electrode. The procedure compares well with atomic absorption results. A modified version of this procedure was sent to Kimberly-Clark. They intended to use the results to obtain a mass balance on paper additives. The autotitrator is currently being repaired.

Several assays for a wide variety of inorganic materials have been developed. These include determination of trace chloride by electrode, sulfate by titration, and a gravimetric phosphate determination. In some cases the assays were inconvenient and results were obtained using techniques such as atomic absorption and colorimetry.

The effects of potassium succinate on the thermal behavior of various minerals was investigated using Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). The comparison of pure samples (dolomite, magnesium carbonate hydroxide, mm calcium carbonate and Omega calcium carbonate) versus samples with 5% and 10% added potassium succinate, gave no conclusive evidence of the effects of potassium succinate on the thermal behavior of the minerals.

Thermal behavior of thirteen carbon-iron oxide samples was determined using TGA. Most samples showed multiple transitions of both weight gain by oxidation and weight loss by combustion.

TGA was used to find the thermal decomposition characteristics of glucovanillin and six related compounds for Project Ambrosia. When analyzed in nitrogen atmosphere, three of the compounds showed multiple weight loss transitions, while the other four showed a single step weight loss transition.

The evaluation of Inductively Coupled Plasma (ICP) spectrometers was completed. A recommendation was made to purchase a Thermo Jarrell Ash Atomscan 25 Sequential ICP.

Investigation into the availability of instrumentation for the determination of Hg was initiated. Baxter Laboratories markets a Colman instrument which utilizes the Hatch and Ott cold vapor procedure. A self-contained analyzer such as this would eliminate setting up a cold vapor system on the atomic absorption (AA) spectrometer.

D. **Plans**: Evaluation of the paper method will continue. Support to other R&D projects for elemental analysis will continue. Paperwork for the purchase of the ICP will be completed. We will try and arrange for a demonstration of the Hg analyzer from Baxter Laboratories.

PROJECT NUMBER: 1902
PROJECT TITLE: Cell/Tissue Culture Research
PROJECT LEADER: I. L. Uydess
WRITTEN BY: J. B. Jones
PERIOD COVERED: May, 1989

## I. TOBACCO MICROBIOLOGY - ART STORAGE STUDY

A. **Objective**: To develop methods and to evaluate the microflora resident in tobacco materials.

B. **Status**: Five cigarette models were fabricated from DL strip blend in the OC Semi-works (C. Rowe) to support an 8-week ART storage study designed by W. Hempfling and R. Carchman. The first two models were made from the original, non-extracted DL strip blend ± humectants and aftercut. The final three models were assembled from this same blend after supercritical extraction. All models were constructed to equivalent firmness and placed into desert (43°C/15% RH) and jungle (43°C/85% RH) rooms for a period of 8-weeks. Additional samples of each model were frozen (-80°C) immediately after production as well as on each sample date to serve as controls. As of the time of this writing, 0-time, +2 day, +7 day, and +2 week samples had been withdrawn from each of the aforementioned storage conditions and analyzed for bacteria, yeast and mold by members of Project 1902. Parallel samples were also distributed on each sample date to members of the Analytical Research Division (B. Handy) and to the Cigarette Testing Laboratory at the OC (E. Wickham). The data from the microbiological portion of this investigation will not be available until the conclusion of the study (July-August, 1989).

C. **Conclusions**: None to be reported at this time.

D. **Plans**: Additional samples for microbiology, chemical and CI testing will be evaluated at 1-2 week intervals through the week of July 2, 1989.

E. **Reference**:

Chadick, D. Notebook No. 8825, p. 6.

## II. ALTERNATE PRESERVATIVES PROGRAM - PHASE I SCREENS

A. **Objective**: To develop procedures and to conduct microbiological screens to evaluate nature-identical preservatives as replacements for and/or as adjuncts to propylparaben.

B. **Status**:

1. The antimicrobial activities of valerophenone, 3-phenyl,1-propanol, β-cyclocitral, β-homocyclocitral, 2-decanone and 1-phenyl,1-propanol were evaluated in the Phase I agar inclusion

screen in order to determine their potential use as preservatives in tobacco. However, while β-homocyclocitral was observed to be marginally effective down to 100 µg/ml (resulting in partial inhibition of growth), none of the aforementioned compounds were as effective in this assay as (a) 100 µg/ml decanoic acid, (b) β-cyclocitrylidine acetic acid (β-CAA), or (c) the 500 µg/ml propylparaben control. (Decanoic acid and β-CAA each totally inhibited the growth of the target organisms, B. coagulans and B. licheniformis at 100 µg/ml.)

2. Citronellol, previously observed to be effective in the agar inclusion assay, was tested in the Phase I shake-flask assay at 0, 100, 150, 200 and 250 µg/ml against the standard, 150 µg/ml propylparaben control. While 150 µg/ml citronellol completely inhibited the growth of the target bacterium, B. coagulans during the first 225 minutes of this experiment, this dose did **not** prevent B. coagulans from resuming growth and attaining confluency overnight. Doses of 200 & 250 µg/ml, however, totally inhibited the growth of B. coagulans over the entire 24 hour period of this experiment.

3. The efficacy of Na-tetrahydrocitrylidine acetate (prepared by R. Izac) was compared to that of its parent compound, β-cyclocitrylidine acetic acid (β-CAA) in the Phase I shake-flask assay. Although 100 µg/ml of each compound inhibited growth of the target organism over the initial 225 minute period of the experiment, only the sodium salt inhibited growth overnight (the acid permitted marginal growth to occur between 225 minutes and 24 hours). At a slightly higher concentration (150 µg/ml), each compound inhibited growth completely over the entire 24 hour duration of this screen.

C. **Conclusions**: None to be reported at this time.

D. **Plans**:

1. Retest citronellol in the Phase I shake-flask screen.

2. Evaluate citronellol and Na-tetrahydrocitrylidine acetate in SEL in the Phase III fermentor screen.

3. Schedule a C-Pilot Plant RL production run ± β-cyclocitrylidine acetic acid for the purpose of subjective evaluations.

E. **Reference**:

Tenhet, S. W. Notebook No. 8281, p. 112.

## III. SALMONELLA/MICROSOME (S/M) ASSAY - CALCIUM EFFECT

A. **Objective**: Varying amounts (0.9% - 6.9%) of calcium acetate were applied to burley CEL (BuCEL) and oversprayed onto bright baseweb (BrBW) to determine:

1. the possible effects on IT CSC delivery and/or burn rate,

2. the effects on S/M IT CSC specific activity (S.A.) when the total solids add-on weight remains constant at 45%.

As a control, various amounts of total solids were added to BrBW (no calcium added) to determine the affect on S/M IT CSC S.A. as compared to the normal 45% add-on level.

B. **Status**: The above work has been completed and the data statistically analyzed.

C. **Conclusions**:

1. The IT CSC delivery **decreased** as the percent added calcium increased.

2. Overall, there was no indication (specific trend) that S/M IT CSC activity was altered by the different levels of calcium added. Unlike previous calcium add-on experiments, some of the test samples were lower in S.A. than their respective controls (although not all significantly). This was apparently due to the amount of Bu CEL in the control samples rather than an affect of added calcium.

3. S/M activity **increased** as the level of total solids added to the baseweb increased (in the absence of any added calcium).

D. **Plans**: No additional experiments have been planned in this area at this time.

E. **Reference**:

Thompson, L. H. Notebook No. 8731, pp. 94-121.

PROJECT NUMBER: 1904
PROJECT TITLE: Tobacco Biochemistry
PROJECT LEADER: D. J. Ayers
WRITTEN BY: T. T. Yu
PERIOD COVERED: May, 1989

## I. LOW NICOTINE STUDY

A. **Objective:** To investigate the biochemistry of the nicotine biosynthetic pathway at the putrescine N-methyltransferase (PMT) step and specifically to isolate PMT from tobacco root extracts.

B. **Status**: PMT activity was determined in leaf tissue collected from group 17 plants. These plants were topped on March 21, 1989. The leaves were collected at 0, 24, 48 and 168 h after topping. Little or no PMT activity was detected in these leaf samples (1).

Work continued with eluting portions of M-6 (PMT active fractions pooled from phenyl-Sepharose) with 1 mM SAM on S-adenosylhomocysteine (SAH) column to further purify PMT. This particular SAH column was discontinued because it exhibited substantially reduced flow rate after 3 portions (100 ml each) of M-6 were eluted. The reason for reduced column flow is being investigated. A new SAH column was packed with fresh gel material. It is being used to elute M-8 post phenyl-Sepharose material. PMT active fractions from SAH column are stored at -80°C for future use (2).

A PMT enriched sample was applied and PMT activity was eluted in sequence from a TosoHaas DEAE-NPR and Poly LC polypropylaspartamide hydrophobic interaction (HIC) columns. The PMT active fractions from the HIC column were concentrated and subjected to SDS-PAGE to reveal the protein banding pattern. The results showed that polypeptides were evident in the 50-70 kD area (3).

The results obtained from an HPLC experiment conducted using a hydroxyapatite (HA) column (Bio Rad) indicated that this column is capable of binding PMT and subsequently releasing it when the column is eluted with a phosphate gradient (3).

The results of protein assay investigations have been summarized. The sensitivity for each method has been defined with samples containing PMT. The protein gold assay method has been recommended for determining protein concentration in dilute samples. The detecting limit of this method is 0.2 µg/ml. At this concentration, 100 µl of sample is needed to run the assay (4).

C. **Plans**: Harvest plants in group 18. Obtain 40-65% ammonium sulfate fraction of root proteins and store at -80°C for future use. Collect leaves from this group of plants; store at -80°C for future use. Continue to process M-8 through SAH column for use in other areas of program. Continue HPLC examination for PMT identification. Analyze a dilute PMT sample containing a standard protein to confirm sensitivity in our matrix and ensure linearity of the method over the appropriate range.

D. **References**:

1. Lyle, J. Notebook No. 8397.
2. Mooz, E. D. Notebook No. 8803.
3. Nakatani, H. Y. Notebook 8384.
4. Yu, T. Notebook 8806.

PROJECT NUMBER: 2106
PROJECT TITLE: Cigarette Performance and Design
PROJECT LEADER: R. W. Dwyer
PERIOD COVERED: May, 1989

I. CIGARETTE DELIVERY MODELING (J. Kao, D. Leister, and B. Dwyer)

A. Objective: Develop computer programs to assist in designing cigarettes.

B. Results: The cigarette-design program has been refined to account for the effects of design on mass burn rate and static burn time. The program now allows the user to select the type of wrapper ash-conditioner and its level of application. With this information and the rod circumference, density, shred cut-width, and wrapper permeability, the program calculates the MBR and SBT. The program has also been modified to predict nicotine and CO delivery levels as functions of design.

C. Plans: CTS has developed a protocol for measuring the TPM, tar, nicotine, and water efficiencies of filters at puff volumes ranging from 15 to 45 cc. We have obtained samples of a variety of new filter materials including paper, ca-web, charcoal-on-tow, charcoal-on-paper, and polypropylene. We plan to have CTS evaluate these filters' efficiencies, and we shall modify the design program to include them. Graphics routines are being developed for creating screen-addressable tables and capability graphs for the program.

II. CIGARETTE DELIVERY ANALYSIS (D. Newman, C. Brumberg, and B. Dwyer)

A. Objective: Develop computer models for predicting the deliveries of cigarettes based on their blend compositions.

B. Results: The current cigarette-design model requires that the delivery and physical data exist for one embodiment of a given blend. This data is then modified to predict performance for any other design using this same blend. We are attempting to combine this model with the TLA data-base in order to predict the delivery of a cigarette based on its blend recipe.

C. Plans: A series of experiments are being planned. We shall have blends fabricated from TLA components, cigarettes made, and their deliveries measured. The experiments will include the effects of casings and aftercuts on deliveries. These results will be the basis for a design program which predicts performance based on blend-component contributions.

III. CORESTA CIGARETTE IGNITION PROPENSITY EXPERIMENTS (J. Whidby and L. Goodwin)

A. **Objective**: At the request of the CORESTA Ignition Propensity Study Group, we have been asked to perform experiments following a protocol they have developed. The objective of these experiments is to determine the ignition potential of a set of cigarettes on a series of substrates, both of which the committee has provided.

B. **Results**: A laboratory has been modified and experimental equipment ordered for these experiments. Currently, all of the hardware and software to execute the program have been installed, and are being tested.

C. **Plans**: This is a blind study in which the nature of both the cigarettes and substrates are unknown to us. The experiments will be completed over the next three weeks and the results sent to the CORESTA committee.

IV. **SELECTIVE GAS PHASE FILTRATION** (D. Simpson and G. Keritsis)

A. **Objective**: Evaluate the potential of available materials for selectively filtering gas-phase components from cigarette smoke.

B. **Results**: A literature and patent review has been completed. The information found was evaluated and a summary prepared. A presentation will be presented to members of the Filter R&D Project and the Cigarette Performance and Design Project.

C. **Plans**: Based on the results of this review, a research proposal will be considered for work in this area.

PROJECT NUMBER: 2500
PROJECT TITLE: Fundamental Chemistry
PROJECT LEADER: J. I. Seeman
PERIOD COVERED: May 1989

I. INORGANICS AS NOVEL TOBACCO MATERIALS ADDITIVES (Fournier, Paine, Podraza)

A. Objective: To develop inorganic materials for novel applications for reduced sidestream and enhanced subjectives in cigarettes and for required properties in novel smoking materials.

B. Results and Plans: A commercially available sample of boehmite has been peptized, reprecipitated and submitted to Bob Rogers. Preliminary results indicate that paper made with this material has similar properties to boehmite samples prepared by A. Kallianos, via precipitation of gels prepared using alkoxides.

To study the effects of metals on sidestream reduction and subjectives, initial attempts have been made to incorporate iron into boehmite via an inorganic nitrate route.

Commercial hydrotalcite was found to incorporate substantial levels (up to 50% net weight increase) of both vanillin and ethyl vanillin. Pyrolysis studies are being undertaken to evaluate these materials as flavor release agents.

II. REMOVAL OF NICOTINE FROM AQUEOUS TOBACCO PROCESSING FLUIDS (Howe, Secor, Seeman)

A. Objective: To develop techniques to remove nicotine and other tobacco alkaloids from aqueous tobacco processing fluids to the exclusion of all other components.

B. Results and Plans: Having disqualified BIS as a candidate liquid ion exchanger, we have continued our efforts using LIXs designed and synthesized in-house. A tris(2-ethylhexyl)-derivative of tartaric acid is showing excellent membrane-mediated nicotine extraction efficiencies and excellent solubility properties. This material also improves the partition coefficient for nicotine (organic/aqueous) more effectively than BIS. Studies have shed light on the mechanisms of membrane extraction of nicotine and loss of LIX to the aqueous feed stock. A large scale (>100 g) preparation of our best LIX was completed.

III. CHEMICAL PHYSICS STUDIES OF TOBACCO CONSTITUENTS (Secor, Seeman)

A. Objective: To obtain structural information about important tobacco constituents/flavorants; to develop information on cluster formation and chemical reactions in clusters.

B. **Results and Plans**: A series of four benzyl methyl ethers were prepared and given to E. Bernstein to complete the series of oxygenated aromatic substrates. Plans have been formulated to examine some new potential chemical reactions in clusters. Studies are being performed on various benzylamines, with and without $CO_2$, to provide information regarding the extraction of nicotine but not the minor secondary alkaloids with supercritical carbon dioxide.

IV. **FLAVOR/ODOR CHEMISTRY** (Podraza)

A. **Objective**: To prepare new substances for flavor/odor evaluation.

B. **Results and Plans**: A series of various 3-substituted hexahydro-2(3H)benzofuranones are being prepared as potential coumarin substitutes. A key common intermediate has been prepared in large quantity and additional steps have been completed.

PROJECT NUMBER: 2501
PROJECT TITLE: Smoke Chemistry
PROJECT LEADER: R. Comes
PERIOD COVERED: May, 1989

## I. SIDESTREAM SMOKE

A. **Objective**: Conduct studies on sidestream smoke including: development of methods for collection and analysis of sidestream gas phase and semivolatiles; visibility determinations; analysis of selected materials relating to sidestream odor and irritation; development of proprietary products.

B. **Status**: 1.) Malfunctions with the Tekmar desorber have been resolved. The unit is back in operation and will be used for gas phase analysis of MS and SS smoke of the Anal./Subj. cigarettes (see #4 below). Semivolatile data will also be generated on these models. The Sievers sulfur detector has been received and is being installed for use with the second Tekmar desorber. A cylinder containing a mixture of sulfur compounds has been ordered to serve as qualitative and quantitative standards for this work. 2.) Work has continued on isolation and identification of compounds from the semi-volatile portion of SS smoke. Two columns with progressively thinner liquid phase films have been placed in series. This resulted in an exceptionally good separation of the ethyl acetate silica gel fraction of SS smoke from Monitor 25 cigarettes and resulted in an increase in tentatively identified and catalogued compounds from 317 to 344. Work in the semi-volatile area will center on an investigation of a potential relationship between the amount of waxes in SS smoke and visibility. 3.) The construction of the second 8-port visibility apparatus continues. The hood has been constructed by the shop and will be installed shortly. All other items for final fabrication are on hand. The calibration and validation of the first 8-port system is continuing. A series of cigarettes has been run on successive days to study system stability and reproducibility. The operation of the instrument has been checked with a series of calibrated neutral density filters. All tests to date have shown excellent system operation. 4.) Cigarettes and analytical data (CI) have been received for investigation in the "Analytical-Subjective Low Sidestream Study." These include six models incorporating two blends and three different wrapper types. The $^{14}C$-study to investigate possible distribution and chemical similarities (differences) has begun with the purchase and purity checks on the $^{14}C$-materials, the preparation of filler and the spraying of the manicured tobacco with the labelled sugars. Cigarette preparation and distribution work will commence shortly. These six models have been smoked on the CORESTA 5-port smoker to determine SSTPM. Data analysis is in progress.

## II. SIDESTREAM SMOKING CHAMBER

A. **Objective**: Design and construct an environmentally controlled chamber to measure selected components of sidestream smoke.

B. **Status**: 1.) Construction of the chamber is nearing completion. Some significant modifications to the HVAC equipment were required, primarily due to laboratory space restrictions. Operational parameters are currently being investigated. Operator training should commence shortly. 2.) Most instrumentation is in-house and is ready for installation upon chamber completion. Some vendor assistance and training has occurred and will continue as equipment comes on-line.

## III. MISCELLANEOUS

1. Pyrolysis gc/ms analyses were carried out on fatty acid derivatives of hydrotalcite in support of the paper technology program. A series of nicotine derivatives was investigated in the unextracted nicotine program (Project Art). Routine gc/ms analyses were conducted as requested.

2. Additional modifications and procedural features have been made to the total recovery smoking machine and to $^{14}C$-sample counting.

3. Smoking of the cigarettes to determine SS nicotine delivery has been completed on the 5-port CORESTA apparatus. Data analysis is in progress.

4. The infrared images taken of cigarettes burning in various CORESTA sidestream chambers and in free air have been analyzed. It appears, based on the images that have been analyzed so far, that the "standard" CORESTA chambers which are narrow and made of glass cause the cigarette coal to burn hotter during smoulder.

PROJECT NUMBER: 2520
PROJECT TITLE: Flavor Research
PROJECT LEADER: Y. Houminer
PERIOD COVERED: May, 1989

## I. FLAVOR RELEASE TECHNOLOGY

A. **Objective**: To investigate the synthesis and pyrolysis of various flavor release systems for use in new or improved products.

B. **Results**: The commercialization of glucose menthol carbonate (GMC) is being actively pursued. The GMC production proposal received from Lee Labs has been reviewed. A decision has been made to proceed with the production of 500 lbs. of GMC. Discussion of all production issues with both Lee Labs and PPG (the supplier of the menthyl chloroformate (MCF) starting material) including the storage of MCF, is in progress.

The search for a potential manufacturer of polyMIC continues. The research at Eastman Kodak has as yet not produced an alternative method for the synthesis of the monomer. A meeting with some Kodak personnel is scheduled for late May to review the progress.Bayer AG have recently indicated that they are very interested in working with us as a commercial supplier of polyMIC.

Two new glucose derived systems designed to release vanillin, cinnamaldehyde and ,α-amylcinnamaldehyde have been prepared by reacting glucovanillin with either cinnamaldehyde dimethyl acetal or α-amylcinnaldehyde dimethyl acetal. The two benzylidene type glucosides as well as some other reference compounds were submitted for TGA and pyrolysis/GC/MS to determine their release efficiency as potential sidestream flavorants.

## II. FLAVOR CHEMISTRY

A. **Objective**: To obtain flavors for subjective evaluation and odor profiling. To isolate and identify tobacco components which are sensorially significant.

B. **Results**: Identification of several fractions of Burley tobacco volatiles were tabulated to give a list of 75 compounds. Of these, 39 were either on hand or commercially available. Dilute solution in ethanol have been prepared for all of these available compounds.

## III. MISCELLANEOUS

3-(2,2,6-Trimethylcyclohexane)-propionic acid and its sodium salt were prepared. The sodium salt is being tested as a paraben replacement.

Work on the HPLC separation of alkaloids in tobacco graft samples has been completed and a memo summerizing the results was issued.

Sidestream evaluation of ART cigarettes, injected with free nicotine, for odor/irritation is in progress. Both subjective and analytical data are being collected for different levels of nicotine.

PROJECT NUMBER: 2525
PROJECT TITLE: Paper and Tobacco Research
PROJECT LEADER: G. H. Bokelman
PERIOD COVERED: May, 1989

## I. SIDESTREAM REDUCTION (S. Baldwin, S. Tafur, B. Rogers, G. Newell, P. Suiter and G. Bokelman)

A. **Objectives**: (1) Evaluate machine-made cigarettes containing cigarette papers produced at the University of Maine and (2) prepare and evaluate, for reduction in sidestream smoke, paper handsheets that contain coatings and/or inorganic fillers with different physical and chemical properties.

B. **Results**: In order to follow up on previous encouraging results, additional high-basis weight, mono-layer ("composite") cigarette papers were prepared at the U. of Maine with varying contents of Multifex MM $CaCO_3$ (1). Papers also were prepared there containing the mineral hydrotalcite for subsequent evaluation with machine-made cigarettes.

A memo was prepared which documents the lack of correlation between Kimberly-Clark's analytical results and our own for Trim V papers (2).

Studies were conducted in which the effects of potassium succinate, potassium bicarbonate or potassium carbonate as "fluxing agents" were examined on papers with calcium carbonate or magnesium carbonate fillers. It appears that optimum sidestream reduction may be achieved with ~4-5% weight add on of potassium ($K^+$). Preliminary results from a related study, using magnesium acetate or magnesium chloride as fluxing agents, suggest that comparable sidestream reduction may be achieved with only 2% weight add on of magnesium ($Mg^{++}$).

A $Mg(OH)_2/MgCO_3$ bilayer paper sized with 7% potassium succinate has given very encouraging preliminary results for sidestream reduction.

Papers were prepared containing three fillers (Multifex MM $CaCO_3$, Omega Fine $CaCO_3$, and $MgCO_3$) in both a trilayer and a composite configuration. Both configurations gave a good reduction in sidestream visibility, but the trilayer offered no particular benefit for the fillers examined.

Investigation of the effect of paper basis weight on sidestream visibility was conducted to determine if this parameter alone could be an effective replacement of dual-wrapped configurations. Although, as expected, the heavier papers did burn faster, there was no obvious optimum basis weight for sidestream reduction.

Handmade cigarettes were prepared from cigarette paper handsheets containing "Smellrite" (a proprietary molecular sieve from Union Carbide) either as the sole filler or in combination with Multifex MM $CaCO_3$. Light extinction analyses showed little reduction in sidestream visibility for any of the "Smellrite"-containing papers. Therefore, this filler will not be investigated further

for sidestream visibility, but plans have been made to examine its effects on sidestream odor.

BET surface area analyses were continued for filler samples submitted by A. Kallianos and J. Paine. These included: 1) seven precipitated alumina sol gels made by different procedures both in-house and at New York Polytechnic University; 2) two samples of calcium aluminate; 3) Boehmite Alumina (Union Carbide); 4) alumina-coated $CaCO_3$; 5) two magnesium phosphate samples (Aldrich); 6) acidic and basic forms of $Na-Al-PO_4$ (Stauffer); and 7) vanilla hydrotalcite. In addition, the surface area of Vantage Excel paper was analyzed.

Problems were encountered with the detector/computer response on the single port light extinction apparatus located in the Tower. D. McRae installed a voltage transformer on the Oriel detector to smooth out incoming current fluctuations. Subsequent analysis by R. Greene indicated a big improvement in the standard deviation of the current reading.

C. **Conclusions**: Data obtained from the study of "fluxing agents," coupled with much earlier data compiled by K. Gunst on single tobacco component cigarettes, is strongly suggestive that the major mechanism for sidestream reduction involves "cracking" of cellulose (and other carbohydrates) rather than formation of a confining ceramic ash (by the cigarette paper). It appears that the potassium cation ($K^+$) is a particularly effective "cracking agent."

D. **Plans**: Although our priorities have changed due to increased concerns about the subjective characteristics of cigarette papers containing magnesium compounds, handmade cigarettes will continue to be prepared from numerous types of handsheets for evaluation of sidestream reduction. Emphasis will be placed on handsheets containing soluble calcium and potassium salts. Dual-wrapped handmade cigarettes will be evaluated in which both the pH and ionic concentration of the cigarette paper are adjusted. Also, studies involving the addition of various salts to tobacco filler will be initiated. Finally, additional mono-layer and bilayer cigarette papers will be prepared at the University of Maine.

E. **References**:

1. Bokelman, G. H., memo to R. N. Ferguson, "Results from April Trip to the University of Maine," April 27, 1989.

2. Baldwin, S. D., memo to W. L. Carter, "Analytical Results on Paper Standards for Trim V Paper," May 4, 1989.

II. GREENHOUSE STUDIES (R. Bass, G. West and G. Newell)

A. **Objective**: To maintain the R&D greenhouses, to conduct plant research studies and to provide greenhouse-grown tobacco materials for support of other R&D programs.

B. **Results**: The hydroponic Burley 21 plants of Group 18 for Project 1904 have been grown to maturity. These 52 plants have been topped and a harvest schedule has been prepared.

The daily greenhouse plant production tasks have been done on schedule including the seeding and transplanting of Burley 21, Speight G-28, Coker 319, K-236, N. rustica, N. glutinosa, N. glauca and Oriental.

C. **Plans**: Maintain plant production schedules. Inspect and monitor plant and environmental conditions on a daily basis.

## III. BLEND COMPOSITIONS OF CHELSEA 100 MM (PLAIN AND MENTHOL)

(G. Bokelman, J. Stimler and General Analytical)

A. **Objectives**: Examine the blend compositions of Chelsea 100 mm Plain and Chelsea 100 mm Menthol. These brands are advertised with the claim, "smoke that smells good."

B. **Results**: Chelsea 100 mm Plain and Chelsea 100 mm Menthol were found to have different blend compositions. The differences between the two Chelsea brands tended to parallel the differences between Winston and Salem. Both Chelsea brands were noteworthy for having exceptionally low burley lamina contents and exceptionally high Oriental leaf contents.

C. **Plans**: No further work in this area is planned.

## IV. SUPPORT ACTIVITIES (G. Newell, R. Bass, G. West, S. Baldwin, S. Tafur and P. Suiter)

A. **Objective**: To provide requested assistance for special projects.

B. **Results**: 1.) A substantial quantity of time was devoted to preparation of the Chemical Research display for phase I of the B.O.B. campaign. In addition, more than a total of 100 mature tobacco plants, hydroponic plants or seedlings were provided to other groups, both inside and outside of R&D, in conjunction with this same campaign. 2.) Dozens of tobacco plants of various sizes were provided and maintained for display purposes in conjunction with the annual PM Stockholders and Board of Directors meetings. 3.) Supporting documentation was provided for a patent disclosure, entitled "Treatment of Cured Unaged Tobacco with Monosaccharides." 4.) Separate, special greenhouse tours were provided for Dr. R. Carchman and for a group of PM affiliated Russian visitors who were accompanied by PM Europe officials and PM R&D representatives. 5.) Several members of the group attended a Liquid Scintillation Counting Seminar by Beckman Instruments.

PROJECT NUMBER: 6502
PROJECT TITLE: Environmental Tobacco Smoke
PROJECT LEADER: C. E. Thomas
PERIOD COVERED: May, 1989

## I. MAINSTREAM AND SIDESTREAM SMOKE STUDIES

A. **Objective**: Improve the method for determining the MS and SS deliveries of solanesol in cigarette smoke TPM.

B. **Results**: Recent problems with the sensitivity of the GC procedure for measuring solanesol in ETS-derived particulates have been corrected. It was found that small amounts of toluene used to enhance the solubility of the solanesol in methylene chloride were interfering with the formation of the BSTFA derivative of solanesol. The solvent system was changed to 100% methylene chloride. The sensitivity was increased from 50 µg/ml to 5 µg/ml.

C. **Conclusions**: The GC method for solanesol has been modified to enhance the sensitivity and is now a viable procedure for studying the amount of solanesol in ETS particulates.

D. **Plans**: Recent results on ETS particulates have suggested that solanesol levels on sidestream particulates could vary depending upon whether the smoke was generated from a puffed cigarette or a static-burned cigarette. With the increased sensitivity of the procedure, this effect will be studied.

E. **References**:

Randolph, H., PM Notebook 8799, p. 6.

## II. PROJECT ART

A. **Objective**: Evaluate the effect of addition of selected acids on the ammonia delivery of ART cigarettes.

B. **Results**: Five mg of citric, malic or lactic acids in 15-µl water were applied to the filters of ART cigarettes (D9TC-1, 11-mg model). The MS ammonia deliveries of these cigarettes were compared to untreated controls and to controls with the same levels of water injected on to the filters. Both MS gaseous ammonia (TDL) and total ammonia (ion chromatography) deliveries were measured. The results are shown in the table below.

| Sample | Gaseous Ammonia | Percent Reduction | Total Ammonia | Percent Reduction |
|---|---|---|---|---|
| ART(D9TC-1) | 8.5 µg/cigt | *** | 53 µg/cigt | *** |
| Water | 6.8 µg/cigt | 20% | 37 µg/cigt | 30% |
| Citric | 3.4 µg/cigt | 60% | 29 µg/cigt | 45% |
| Malic | 3.2 µg/cigt | 60% | 27 µg/cigt | 50% |
| Lactic | 3.0 µg/cigt | 65% | 27 µg/cigt | 50% |
| Mon# 25 | 2.3 µg/cigt | *** | 25 µg/cigt | *** |

The results of this study showed a 60% reduction in gaseous ammonia and a 50% reduction in total ammonia for all of the acid-treated samples [1]. This confirmed earlier studies using citric and malic acid [2]. These samples were also analyzed for low molecular weight pyridines and pyrazines in the TPM basic fraction [3]. Citric and malic acids reduced these classes of compounds in the TPM basic fraction by approximately 50% while lactic acid reduced them by 20% versus the controls. No differences in basic compounds were noted for the water-treated filters.

C. **Conclusions**: While the ammonia deliveries of ART control cigarettes are twice the levels of Mon# 25 cigarettes, they are a factor of two below the level of ammonia in smoke that can be detected by a smoker [4]. However, the increased basicity of the smoke increases the deliveries of pyridines and pyrazines in the MS. It has been shown in this work that reducing the MS ammonia deliveries by adding small amounts of organic acids to the filters also reduces the deliveries of these classes of compounds. The increased levels of ammonia and other basic compounds may account for some of the smoke subjectives of the ART product.

D. **Plans**: Further experiments will be conducted using the Mercedes and Muratti 2000 filters in collabration with G. Keritsis [5]. These filters contain silica gel and charcoal respectively and will be evaluated for their removal efficiencies of ammonia and other basic smoke components.

E. **References**:

1. Parrish, M., "Reduction of MS Ammonia of ART Cigarettes with Filters Treated With Citric, Malic, and Lactic Acids", Memo to J. Charles, May 17, 1989.

2. Parrish, M., "Summary of Results and Recommendations for the Reduction of MS Ammonia in Art Cigarettes", Memo to J. Charles, April 11, 1989.

3. Hsu, F., Buckner, M., "Smoke Modification of ART Cigarettes", Memo to M. Parrish, April 25, 1989.

4. Parrish, M., Harward, C., Vilcins, G., "determination of Gaseous Ammonia Thresholds in Cigarette Smoke. Part I. Analytical Methodology"' Completion Report Acc# 85-175, June 1985.

5. Personal Communication, May 1989.

6. Parrish, M., PM Notebook 8729, pp. 64-67.

PROJECT NUMBER: 6505
PROJECT TITLE: Special Investigations/Methods Development
PROJECT LEADER: D. F. Ingraham
PERIOD COVERED: May, 1989

## I. PROJECT ART

A. **Objective**: Provide analytical support to project ART.

B. **Results**:

Two ageing studies are in progress. One study is tracking the nicotine levels in ART filler over time under cold, room temperature, and accelerated ageing conditions. The purpose of the second study, being coordinated by W. Hempfling, is to determine if cigarettes made from ART filler (and four other research models) undergo any change in the nicotine content under conditions of low temperature, desert room, and jungle room. Initial data from both studies are currently being tabulated.

Initial studies showed that the new nicotine method (number E-86A) is suitable for the determination of nicotine in spent stems without modification.

C. **Plans**: Continue analysis of the samples from the two ageing studies. Provide support to the ART processing facility as needed. Finish work on stem analysis and update method.

## II. RESPONSE TO ANALYTICAL REQUESTS

A. **Objective**: To provide analytical support to R&D and Operations personnel and projects.

B. **Results**:

Analyses and investigations by project personnel during the month of May included:

Numerous ETS samples from XAD-4 resin and charcoal traps were analyzed for nicotine and volatile organics, respectively as part of a study on the removal of ETS under conditions designed to simulate a room with high smoking activity. These results have been forwarded to J. Lephardt for interpretation.

Non-routine packaging materials continue to be quantitatively and qualitatively analyzed for residual solvents.

Several packs of various Marlboro brands were analyzed for ethanol for the Marlboro Standardization Program.

Initial work was begun on the development of an HPLC method for the determination of added methoprene in leaf. Stemmery samples analyzed using this method required less cleanup and resulted in

cleaner chromatograms than the standard GC procedure. Much of the improvement is believed to result from the use of a UV detector on the HPLC versus the non-selective FID used with GC.

Various tobacco flavor extracts were analyzed for nicotine content. Results were reported to F. Daylor.

Two customer complaint samples were analyzed this past month. No foreign materials were observed.

PROJECT NUMBER: 6902
PROJECT TITLE: Biochemical Special Investigations
PROJECT LEADER: B. D. Davies
WRITTEN BY: R. L. Dunn
PERIOD COVERED: May, 1989

## I. NICOTINE SPECIFIC MONOCLONAL ANTIBODY

A. **Objective**: To obtain a monoclonal antibody (MCA) against nicotine (NIC-MCA).

B. **Results**: An aliquot of nicotine specific polyclonal serum (PCA), and the documentation on its associated characterization, was shipped to P. Echlin. He has received it and has started immunocytochemical studies to look for nicotine in plant material.

C. **Plans**: Test spent culture media from the newest fusion clones for nicotine specificity.

D. **References**:

Davies, B. D. Notebook No. 8638, p. 190.
Crockett, E. A. Notebook No. 8783, p. 85.

## II. ADDITIONAL APPROACHES TOWARD PUTRESCINE METHYLTRANSFERASE (PMT) ISOLATION

A. **Objective**: Provide additional experimental approaches to assist in the effort to isolate PMT.

B. **Results**: An experiment was conducted to determine if the covalent bond formed when $^{3}H$ is attached to PMT during photoaffinity labeling (PAL), is stable when exposed to several pHs. Portions of $^{3}H$ labeled PMT were incubated with buffers of pH 7, 8, 9, 10, or 13 for 1 hr. at room temperature. Following treatment, the samples appeared to lose ~25-30% of the $^{3}H$ label. An additional experiment was conducted to confirm and extend these observations. Preliminary analyses indicate that incubation at room temperature may effect the loss.

Two experiments were conducted to examime the time course of photostimulated binding of $^{3}H$ to PMT. Analysis of the data indicated that binding increased in a linear fashion up to 2 hr, the maximum time tested.

Two concentrations of putrescine (100 and 250 uM) were compared to SAH (100 uM) in their ability to inhibit photostimulated incorporation of $^{3}H$ into PMT. Analysis of the data indicated the following inhibitions of total binding: SAH-68%; putrescine at 100 uM-24% and putrescine at 250 uM-54%. Putrescine does appear to inhibit PAL.

Data analysis from an incorporation experiment conducted last month have been completed. Following analysis it was determined that the

bulk of the $^{3}H$ was contained in a ~55 Kd protein. The molecular weight of PMT is reported to be in this range.

C. **Plans**: Examine the individual protein labeling profile of putrescine inhibited, PMT-PAL samples. Examine the effects of an extended time course of photolysis (>2 hr.) on PMT-PAL. Continue to examine the causes of loss of $^{3}H$ from labeled samples.

D. **References**:

Crockett, E. A. Notebook No. 8783, p 85.
Davies, B. D. Notebook No. 8638, p. 190.
Dunn, R. L. Notebook No. 8721, p. 80-100.

PROJECT NUMBER: 6906
PROJECT TITLE: Biological Effects of Smoke
PROJECT LEADER: J. M. Penn
WRITTEN BY: W. R. McCoy
PERIOD COVERED: May, 1989

## I. PROTEIN KINASE C (PKC) ASSAY IN INTACT CELLS

A. **Objective**: To examine the response of quiescent 3T3 cells to 12-0-tetradecanoylphorbol-13-acetate (TPA), 2R1 CSC, X6D4CDL CSC and X6D5RN CSC in the PKC intact cell assay.

B. **Results**: In the first experiment performed, the 19.5 hr. treatment doses were 10 and 100 ng TPA/ml, and 20 and 200 mg CSC/ml. The two doses of TPA resulted in approximately the same increase in phosphorylation. The 20 mg/ml doses of CSC caused some increase in phosphorylation, but the 200 mg/ml doses did not. In the second experiment, TPA doses were 1 and 10 ng/ml and CSC doses were 20 and 100 mg/ml.

C. **Conclusions**: Data from the first experiment showed phosphorylation increases due to 10 or 100 ng TPA/ml to be 2 or 3-fold greater than increases due to 20 mg CSC/ml. There were small differences among the CSCs, but whether this is within experimental error remains to be tested. The lack of increase in phosphorylation with the 200 mg CSC/ml treatment was most likely a toxicity effect.

D. **Plans**: Evaluate data from the second experiment to determine the experimental error within the data and to determine if the difference in response among CSCs is greater than data variability.

E. **Reference**:

Nixon, G. M. Notebook No. 8711, p. 98.

## II. CHARACTERIZATION OF JB6 MOUSE EPIDERMAL CELL LINE

A. **Objective**: To implement the JB6 cell transformation assay in our laboratory.

B. **Results**: Several transformation assays were set up according to literature protocol using 10 and 20% serum agar medium. No colonies were observed for the transformed clone RT101. We have learned that there have been a number of protocol changes from the method reported in the literature.

C. **Conclusions**: The literature protocol, which we had been following, was not optimal for producing colony growth in soft agar.

D. **Plans**: We are obtaining a copy of the modified protocol for the transformation assay from which will be tested as soon as it and the special medium required are received.

E. **Reference**:

Nixon, G. M. Notebook No. 8711, p. 98.

## III. ACQUISITION AND MAINTENANCE OF ADDITIONAL CELL LINES

A. **Objective**: To acquire and maintain a variety of cell lines for use in biochemical assays.

B. **Results**: More MT 1/2 cells arrived from the University of Texas. The cells are growing well and one vial of cells was cryopreserved. Work was begun to establish a procedure for immunofluoresence staining of keratins. Preliminary results are encouraging.

C. **Plans**: Plans include mycoplasma testing, a growth curve experiment, and additional innumofluorescence experiments with expanded controls.

D. **Reference**:

Patskan, G. J. Notebook No. 8751, p. 199.

## IV. INHIBITION OF EGF BINDING ASSAY

A. **Objective**: To evaluate catechol depleted CSC in the EGF assay.

B. **Results**: Catechol-depleted CSC was less active than the control CSC.

C. **Plans**: To scale-up and improve chromatographic removal of catechol from CSC.

D. **Reference**:

Vaughan, B. G. Notebook No. 8537, p. 199.

## V. PDBU BINDING ASSAY

A. **Objective**: To examine the effects of TPA and 2R1 CSC on the binding of $^{3}$H-PDBu.

B. **Results**: Two $^{3}$H-PDBu experiments were performed at time of binding. Treatment plates were incubated with 2R1 CSC and TPA for two hours on ice, washed three times with binding buffer, hot and cold PDBu were added, and plates were incubated on ice for: (1) 5, 10, and 15 minutes; and (2) 5, 15, and 30 minutes. Results of the first experiment showed no significant change in binding for all treatments with time. However, TPA did show normal inhibition.

The second experiment indicated a stimulation of binding with 2R1 CSC treatment (as much as 100%), while TPA showed inhibition as normally seen.

C. **Plans**: To complete a $^{3}$H-PDBu inhibition (preincubation time 19.5 hours) experiment which is in progress.

D. **References**:

Burruss, T. J. Notebook No. 8804, p. 82.

PROJECT NUMBER: 6908
PROJECT TITLE: Smoke Condensate Studies
PROJECT LEADER: A. H. Warfield
PERIOD COVERED: May, 1989

## I. ORIENTAL TOBACCO STUDIES

A. **Objective**: To determine if the inhibition of TSNA formation/pyrosynthesis observed for oriental tobacco is due to agronomic effects.

B. **Results**: Complete analytical data were obtained from ARD on eight oriental varieties. Wide ranges in the levels of various components were observed depending on the country of origin as well as grade designation in some cases. GMU (Greek Macadonia Unique) was found to have the highest levels of total alkaloids, total nitrogen, nitrate nitrogen and soluble ammonia. Starch varied considerably between the grades of Turkish Izmir and appeared to correlate positively with reducing sugars. Significant variations were found with respect to β-methyl valeric acid, K/Ca ratio, and total dicarboxylic acids. Replicate filler TSNA analyses were completed on the eight varieties. Only TIK (Turkish Izmir Kapa) and GMU had levels of endogenous TSNA typical of those found in typical blended oriental fillers. Very low endogenous TSNA values were observed for the other varieties. The latter two varieties as well as TIU (Turkisk Izmir Unique) were chosen for MS TSNA analysis. GMU was found to yield the highest level of MS TSNA when the data were calculated on either a gram filler consumed or TPM basis, despite the fact that TIK had the highest levels of endogenous TSNA.

C. **Plans**: Filler and MS TSNA data will be correlated with the available analytical data on the eight oriental varieties. An attempt will be made to evaluate the individual varieties with respect to their likely level of inhibition of MS TSNA.

D. **Reference**:

Keene, C. K. Notebook No. 8754, p. 159.

## II. ORIENTAL INHIBITOR STUDIES (CEL)

A. **Objective**: To determine the causative agent(s) responsible for the reduction in MS TSNA observed when oriental (Or) CEL is added to burley (Bu) CEL and applied to Bu base web (BW), relative to a control RL prepared from BuCEL on BuBW.

B. **Results**: An RL has been prepared from a mixture of Br and Bu CELs and applied to BuBW to determine whether this RL delivers less MS TSNA than the RL prepared with the same level of BuCEL alone. This would be analogous to the results obtained by mixing OrCEL with BuCEL and preparing an RL using BuBW, where a reduction in MS TSNA was obtained. In the latter case it was hypothesized that

the sugars in the OrCEL reacted with the ammonia in the BuCEL to generate an inhibitor for TSNA pyrosynthesis or transfer. The sugar-amine hypothesis was proposed because of the similar reduction in MS TSNA afforded by mixing sugars with BuCEL and applying to BuBW. However, the Br/BuCEL on BuBW RL yielded no reduction in MS TSNA relative to a BuCEL on BuBW control. It is apparent that the combination of the sugars present in BrCEL and the ammonia in BuCEL under the conditions of this experiment did not generate an inhibitor capable of reducing MS TSNA.

C. **Plans**: No further experiments on BrCEL/BuCEL mixtures are planned. Data collection on the BuCEL/OrCEL/"sugars" on BuBW aging study will continue. A melanoidin will be prepared from glycine and glucose, dialyzed against distilled water, and evaluated as an additive to an extracted burley filler for the purpose of reducing MS TSNA. It is expected that a high molecular weight material such as this will release pyrolysis products at high temperatures, some of which may act as inhibitors of pyrosynthesis or transfer that occurs at high temperatures.

D. **Reference**:

Morgan, W. R. Notebook No. 8579, p. 178

## III. ORIENTAL INHIBITOR STUDIES (ORGANIC EXTRACTS)

A. **Objective**: To determine whether the causative agent(s) responsible for the reduced levels of MS TSNA observed for Or tobacco can be removed with organic solvents and applied to other fillers as a means of decreasing the MS TSNA levels delivered by these fillers.

B. **Results**: Data reported in April showed that reductions of 34, 30, and 11% were obtained for MS NNN, NAT, and NNK, respectively, for 5% EtOH/hexane extracted Bu filler treated with a methylene chloride (MeCl) extract of blended Or tobacco. These results were obtained by applying the extract shortly after it was prepared. Application of a sequential methanol (MeOH) extract of the same Or tobacco gave a slight increase in MS TSNA relative to control. After 1-2 months of standing at room temperature, the same extracts were reapplied to the extracted Bu filler and resmoked for MS TSNA. In this case, the MeCl extract did not cause a reduction in MS TSNA as observed before. The reason for this discrepancy is unknown, although a possible explanation is that a component in the extract became oxidized on standing. The reason for the increase in MS TSNA as a result of applying the MeOH extract is presumably because the MeOH extract contains the minor alkaloids from the Or filler, which would generate additional pyrosynthetic TSNA. It has been shown that MeCl is a poor extraction solvent for minor alkaloids.

C. **Plans**: A hexane extract is being prepared from Or filler. This will be followed by a sequential MeCl extraction, and both extracts will be evaluated as MS TSNA inhibitors. If data are

promising a large sample of Or filler will be extracted using supercritical $CO_2$ to produce an extract to be fractionated in attempts to isolate and identify the inhibitor.

D. **References**:

Haut, S. A. Notebook 8768, p. 125.

## IV. MISCELLANEOUS AND SUPPORT STUDIES:

A. **Objective**: To conduct studies of the TSNA content of filler and/or MS smoke as necessary to support other PM programs.

B. **Results**: Filler, MS and SS TSNA data were obtained on zero-time samples for an ART aging study.

C. **References**:

Morgan, W. R. Notebook No. 8579, p. 178
Tickle, M. H. Notebook 8716, pp. 177-180.

PROJECT NUMBER: 6912
PROJECT TITLE: Tobacco/Smoke Relationships
PROJECT LEADER: S. B. Hassam
PERIOD COVERED: May, 1989

## I. TSNA PRECURSORS

A. **Objective**: To determine the precursors of MS TSNA.

B. **Results**: Thin layer radiochromatography (TLRC) of MS TPM extracts from radiolabeled nicotine cigarettes was continued. As described in the April monthly summary, the MS TPM buffer solutions were extracted with methylene chloride. Aliquots of the original buffer extracts from the two smoking runs and the methylene chloride extracts were each chromatographed on analytical silica gel plates in two solvent systems: (1) acetonitrile (2) acetonitrile/methanol/15M ammonium hydroxide (20/1/1 or 20/1/0.25). Using a scanning time of 60 min per sample, the radioanalyses indicated that a major portion (estimated at ~70% for the methylene chloride extracts) of the radioactivity chromatographed with nicotine. The remainder of the radioactivity migrated as a streak of minor components, including a region that cochromatographed with standard $^{14}$C-NNK. These minor components were not easily detectable with sample applications of less than 0.5 nCi or with scanning times of 10 min per sample. A standard solution of ~64 pCi of $^{14}$C-NNK was applied as the lower limit of detection. Additional data processing is in progress.

Preparative chromatography of the methylene chloride extracts (smoking #1, 136 nCi; smoking #2, 151 nCi) was done on silica gel plates (1000 μ thickness) in methylene chloride/methanol (20/1). A portion of the smoke sample spiked with $^{14}$C-NNK was applied as reference. The plates were scanned to locate radioactive regions. Three broad zones were isolated: (1) a polar zone, which had the major portion of the applied radioactivity associated with it; (2) a less polar zone, including the region co-chromatographing with NNK; (3) the remainder of the silica gel, up to the solvent front. TLC of zone 1 indicated the radioactivity to be $^{14}$C-nicotine and other components of similar polarity. The radioactivity in zone 2 was found to migrate as a streak of components, including a region that cochromatographed with standard $^{14}$C-NNK. No radioactive components were detected in zone 3. Liquid scintillation analyses of these samples is in progress. Additional processing of the TLC data is in progress.

TLC analyses were done of the aqueous phases generated after methylene chloride extraction of the MS TPM buffer solution. In the extracts of interest, the results indicated $^{14}$C-nicotine as essentially the only $^{14}$C component in these samples.

Brief analyses of the SS TPM buffer solutions showed a chromatographic profile very similar to that of MS TPM.

Nonradiolabeled cigarettes of the same filler blend and configuration obtained from CRD were smoked and analyzed for TSNA by Project 6908.

C. **Plans**: Complete thin layer radiochromatography of samples isolated by preparative TLC. Complete LSC of samples. Analyze samples by reverse phase HPLC and/or TLC radiochromatography and by GC/TEA.

D. **Reference**:

Hassam, S. Notebook No. 8823, pp. 16-17.

## II. CROSSED SOLUBLES/BASE WEB STUDY (CHEMISTRY)

A. **Objective**: To investigate the smoke chemistry of model cigarettes made from all possible combinations of solubles from bright, burley and oriental tobaccos on base webs from the three tobaccos.

B. **Results**: Thirty-four filler types prepared using bright base web and modified bright CEL were made into cigarettes. The bright CEL contained varying amounts of our standard protein, an amino acid mixture or ammonium acetate. Many of the cigarettes had objectionable odors associated with the smoke and in some cases the cigarettes would not burn. For these reasons, the number of smokings were limited to one for some of the cigarettes. The CSC from each smoking was dissolved in DMSO for S/M assay. Smoking of control cigarettes is in progress in collaboration with Project 6908.

Samples of burley CEL treated previously with various exchange resins were dissolved in water, in preparation for spraying on bright base web. The major types of sample, pH, dry weight, and the concentration in water are as follows: (1) Bio-Rex 70 cation exchange resin-treated CEL, pH 5.5, 51.0 g, 0.4 g/mL; (2) Ion retardation resin AG11A8-treated CEL, pH 6.6, 64.6 g, 0.56 g/mL; (3) Chelex 100 resin-treated CEL, pH 5.4, 29.6 g, 0.59 g/mL. In addition, a sample (pH 1.5, 22.1 g, 0.20 g/mL) was also prepared from CEL treated previously with a strong cation exchange resin by David Williams.

With assistance from Gus Keritsis, the electrodialysis unit was tested by project 6912 members. An incorrectly installed cable led to a blown fuse. Prompt troubleshooting and repairs by Aubrey Burton enabled the testing to be completed. The unit is now operational.

C. **Plans**: Preparation of fillers and cigarettes will be continued. Cigarettes will be smoked for S/M assay. Formulation of plans for C Pilot Plant production of RL will be continued. Preliminary experiments to demineralize CEL using the electrodialysis unit will be initiated.

D. References:

Hellams, R. Notebook No. 8613, p 160.
McGee, N. Notebook No. 8743, pp. 43-50.
Drew, S. Notebook No. 8800, pp. 25-30.
Hassam, S. Notebook No. 8712, p. 16-17

## III. DEVELOPMENT AND APPLICATION OF ANALYTICAL PROCEDURES

A. Objective: To develop, maintain and apply analytical methodology for minor alkaloids, PAHs and other compound classes.

B. Results: Thirty-six samples of extracts of bright filler obtained from a multiple pass system were analyzed for minor alkaloids and nicotine by capillary GC/NPD.

The efficacy of ascorbic acid as a filter additive in reducing formaldehyde deliveries was investigated using 2R1F cigarettes. Ascorbic acid was added to the CA filter (plug/space/plug) at levels of 30 and 150 mg. No significant reduction in delivery of formaldehyde was observed.

C. Reference:

Levins, R. J. Notebook No. 8824, p. 20.

## IV. SUPPORT FUNCTION: CONDENSATE PREPARATION

A. Objective: To fabricate cigarettes, perform smokings, and prepare condensate as needed for biological and chemical analysis.

B. Results: Fourteen cartons of Marlboro 100 cigarettes were smoked on a 30-port Borgwaldt smoking machine to collect CSC for fractionation studies at the request of Sherman Lin. Handmade cigarettes were prepared for Project 6908.

C. References:

Hellams, R. Notebook No. 8613, p. 160.
McGee, N. Notebook No. 8743, pp. 42, 52.

PROJECT NUMBER: 8101
PROJECT TITLE: Cigarette Testing Services Division
SECTION LEADER: Jane Y. Lewis
PERIOD COVERED: May, 1989

## I. MARKET ACTIVITY

A. **Objective**: To monitor and report new brand introductions and brand modifications for the domestic and international cigarette markets.

B. **Results**:

1. Domestic

Philip Morris is test marketing Marlboro Ultra Lights 85 and 100 cigarettes. Marlboro Ultra Lights in a red box with cork tipping is being tested in Portland, Oregon; Marlboro Ultra Lights in a blue box with white tipping is being tested in Indianapolis, Indiana. These cigarettes deliver 6 mg tar and 0.5 mg nicotine.

R. J. Reynolds has decreased the ventilation (40 to 32%) of Doral Lights Menthol 100 cigarettes. This resulted in a higher CO delivery (11 to 13 mg) and a higher total and filter RTD. A lower nicotine delivery (0.8 to 0.7 mg) correlated with lower total alkaloids.

2. International

Japan Tobacco, Inc. introduced Cabin Super Mild King Size cigarettes in Japan. This brand delivers 10 mg tar and 0.8 mg nicotine and is similar in smoke deliveries and blend to Cabin Mild King Size.

## II. INDUSTRY MONITOR

A. **Objective**: To produce a monitor cigarette (four million units) to be used as an industry-wide monitor for smoking analyses (TPM, nicotine and water).

B. **Results**: Four models for the industry monitor were made in Semiworks. The standard deviation on the weight was tighter than normal factory procedures. The cigarettes were tested for firmness, tobacco weight, RTD, tar and nicotine. One of the models was selected and is to be remade in Semiworks. The tobacco weight was increased by 10 mg in this model to increase firmness.

C. **Plans**: Marlboro blend with Cambridge aftercut will be used for the four million industry monitor samples. These cigarettes will have 27 mm tipping, and a firmness target of 2.8 mm. The four million cigarettes are scheduled to be made in Semiworks beginning June 6.

## III. COMPARISON OF SMOKING PROCEDURES

A. **Objective**: To establish the relationship between tar and nicotine values generated by different smoking procedures used throughout the world.

B. **Results**: Correlation curves for tar and nicotine data were generated for the FTC, ISO, UK, Canadian and Australian smoking methods. The brands tested were Merit Ultra Lights, Merit, Marlboro Lights, Marlboro and Newport.

C. **Plans**: Curves for tar and nicotine will be generated for the TIOJ and DIN methods.